Abstract

Photoperiod is one of the most important environmental signals that plants perceive and use to control their flowering time. In Arabidopsis, *CONSTANS* (*CO*) plays a key role in the photoperiod regulation of flowering by upregulating the floral promoter *FLOWERING LOCUS T* (*FT*) under inductive long days (LDs). Overexpression of *CO* causes photoperiod-independent early flowering and is dominant over the repression of flowering by the vernalization gene *FLOWERING LOCUS C* (*FLC*). Barley carries two close orthologs of *CO*, *HvCO1* and *HvCO2*, but its main photoperiod response gene is *Ppd-H1*. *Ppd-H1* promotes flowering by upregulating the barley ortholog of *FT* (*HvFT1*) under LDs, but *HvFT1* induction is counteracted by the LD floral repressor *VERNALIZATION 2* (*VRN-H2*). Overexpression of *HvCO1* has been shown to upregulate *HvFT1* and to accelerate flowering time in barley, suggesting a degree of conservation in the *CO-FT* regulon. Nevertheless, it is not yet known how *HvCO1/HvCO2*, *Ppd-H1* and *VRN-H2* interact to regulate *HvFT1* under LDs. On the other hand, HvCO1/HvCO2 and Ppd-H1 are not functional under short days (SDs) and *HvFT1* is not expressed. Promotion of flowering under SDs is attributed instead to *HvFT3*, the candidate gene for the *Ppd-H2* locus. While *HvFT1* initiates floral transition at the shoot apical meristem (SAM) through the upregulation of the *APETALA1/FRUITFUL* (*AP1/FUL*)-like gene *VRN-H1*, the effect of *HvFT3* on shoot apex development has not yet been elucidated. A less significant effect of *HvFT3* on flowering time under LDs was also reported, but this effect is highly influenced by the main photoperiod and vernalization genes.

The objectives of the first part of the present work were to functionally characterize *HvCO2* and to examine potential genetic interactions among *Ppd-H1*, *VRN-H2* and *HvCO1/HvCO2* and their effects on flowering time in barley. In the second part, we aimed to investigate the role of *HvFT3* in the regulation of vegetative and reproductive growth in barley under LD and SD conditions and to characterize the genetic interactions of *HvFT3* with *Ppd-H1*, *VRN-H1* and *VRN-H2* as a mean to elucidate the role of *HvFT3* in the promotion of flowering under LDs.

HvCO2 and *HvFT3* were overexpressed under the maize ubiquitin promoter in the spring barley Golden Promise and flowering time and expression of main flowering time genes were analyzed. Additionally, vegetative and reproductive development of the SAM were compared in transgenic and control *HvFT3* plants under SDs and LDs. Flowering time and gene expression were investigated in F_2 populations derived by crossing each of the transgenic lines *Ubi::HvCO2* to the winter barley Igri to study interactions of *Ubi::HvCO2* and of *Ubi::HvFT3* with the natural variation at *Ppd-H1, VRN-H1* and *VRN-H2*. Observed genetic interactions involving *Ubi::HvCO2* were compared with those of *Ubi::HvCO1* in an independent F_2 population *Ubi::HvCO1* x Igri, whereas *Ubi::HvFT3* interactions with *Ppd-H1* and the vernalization genes were confirmed using non-transgenic F_4 families segregating for the functional variation of *HvFT3*.

HvCO2 overexpression induced *HvFT1* expression and accelerated flowering time in spring genotypes, however, *HvFT1* induction and day-length sensitivity were mainly controlled by functional variation at *Ppd-H1*. Furthermore, *Ubi::HvCO2* reduced the delay in flowering time of winter genotypes but simultaneously upregulated the floral repressor *VRN-H2* under LD, and even under SD when *VRN-H2* is typically not expressed. Functional variation at *Ppd-H1* in turn, controlled expression of *VRN-H2* in non-transgenic F_2 plants under LDs. These results suggest that both floral activators *HvCO2* and *Ppd-H1* indirectly repressed flowering before vernalization by controlling expression of *VRN-H2* under LDs.

Analysis of the *Ubi::HvFT3* lines revealed that overexpression of *HvFT3* accelerated the floral transition and the early reproductive development of the shoot apex independently of the photoperiod. However, *Ubi::HvFT3* failed to promote inflorescence development during the late reproductive phase under SD and thus could not compensate for the lack of *HvFT1* under non-inductive conditions. *Ubi::HvFT3* induced the floral transition by upregulating *VRN-H1* in the leaves and at the shoot apex. *VRN-H1* upregulation in turn led to the downregulation of *VRN-H2* and thus abolished the vernalization requirement of winter genotypes. Additionally, *Ppd-*

H1 modulated the effect of *Ubi::HvFT3* and *HvFT3* on flowering time under LD in transgenic and wild type plants, respectively.

The present study thus revealed new points of convergence of and within the photoperiod and vernalization pathways which might provide a better understanding of the complex interactions of genes controlling flowering time in temperate cereals.